

Innovations

The Fungus among Us: Use of White Rot Fungi to Biodegrade Environmental Pollutants

The recognition that environmental pollution is a worldwide threat to public health has given rise to a new, massive industry for environmental restoration. For both economic and ecological reasons, biological degradation has become an increasingly popular alternative for the treatment of hazardous wastes. Recently, white rot fungus, the only organism that degrades wood, was shown to exhibit unique biodegradation capabilities.

Hazardous waste remediation has become a serious financial problem for companies that have failed to adequately address pollution problems in the past. Superfund site cleanup costs alone can range from \$10 to \$50 million per site. The total cost for remediation of hazardous waste in the United States is estimated between \$0.5 and \$1 trillion. Use of organic clean up techniques may significantly reduce these costs. In addition, the number of chemicals that can be biodegraded is constantly increasing as organisms are engineered to degrade toxins.

Lignin and Fungi

Lignin is a natural polymer of the cell wall that gives strength to wood. White rot fungi, which use cellulose as a carbon source, possess the unique ability to degrade lignin completely to carbon dioxide to access the cellulose molecule. Although this ability has been recognized for many

years, only recently have investigators begun to understand the mechanisms by which this degradation is accomplished. Scientists hope that an understanding of how white rot fungus degrades wood will lead to its successful application in hazardous waste remediation.

The lignin degradation enzyme system of white rot fungi is extracellular and unusually nonspecific. Peroxidases and hydrogen peroxide, which are secreted by the fungi, catalyze reactions of the highly reactive and nonspecific free radicals, resulting in the depolymerization and degradation of lignin. Although lignin is naturally a highly oxidized polymer, it can eventually be completely oxidized to carbon dioxide by white rot fungi. Understanding how this is accomplished is central to understanding how many highly oxidized environmental pollutants can be degraded by the fungi. The extracellular biodegradation system also explains why the fungi can be quite resistant to toxic or mutagenic chemicals.

Environmental Pollutants

The growing list of chemicals white rot fungi are able to degrade includes many pesticides, polyaromatic hydrocarbons, PCBs and other halogenated aromatics (including dioxins), some dyes, TNT and other nitro explosives, and other toxic chemicals such as cyanides, azide, carbon

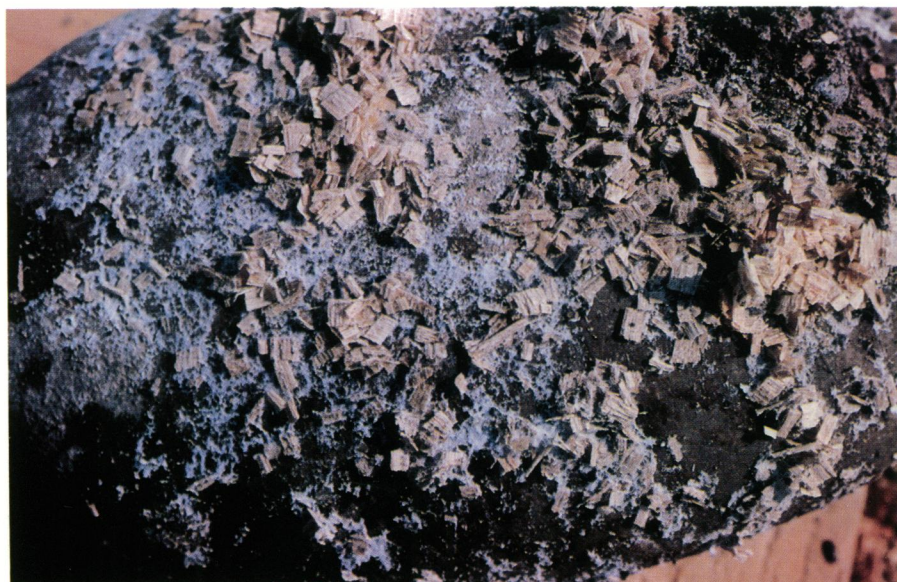
tetrachloride, and pentachlorophenol. White rot fungi are also nonselective in degrading all the chemical components of complex mixtures. For example, all the components of Aroclors (PCB), toxaphene, creosote, and coal tars are degraded by the fungi. In some cases this ability is related to the nonspecific nature of the peroxidases secreted by the fungi; in other cases it is related to the variety of mechanisms that the fungi use to degrade chemicals.

Mechanisms of Biodegradation

The first published report on the degradation of chemicals by white rot fungi demonstrated that these fungi degrade DDT, PCB, Lindane, dioxin, and benzo[*a*]pyrene. That initial publication not only demonstrated the nonspecificity of the fungus but also showed its ability to oxidize highly chlorinated chemicals. Such highly chlorinated chemicals are electron deficient due to the high electronegativity of the chlorine. These chemicals must therefore be reduced before they can be oxidized.

White rot fungus synthesizes and secretes a substrate for its own peroxidase enzymes: veratryl alcohol (3,4-dimethoxybenzyl alcohol). The partially oxidized veratryl alcohol (the cation free radical) then oxidizes other chemicals that are not directly oxidized by the peroxidase enzymes.

To complete further degradation, the fungus produces organic acids that also inhibit veratryl alcohol oxidation. Why would the fungus produce a substrate and an inhibitor of its own enzymes? Further research demonstrated that oxalate, the major organic acid synthesized by white rot fungi, is also an excellent inhibitor of lignin peroxidases. Oxalate is easily oxidized to carbon dioxide, thus its oxidation is essentially irreversible. However, the oxidation of oxalate is a two-electron oxidation, whereas the reduction of the veratryl alcohol cation radical consumes only one electron. Therefore, the odd electron left in oxalate is available for other reductions. A number of electron acceptors can thus be reduced by lignin peroxidases provided with hydrogen peroxide, veratryl alcohol (which is now called a "mediator"), and oxalate (which has been termed the "donor"). The reductive dechlorination of carbon tetrachloride (a highly oxidized chemical) to the trichloromethyl radical was demonstrated with this system. The reductive dechlorination process was accomplished using a peroxidase, thus opening up a new field of investigation



Steven Aust

What rot! White rot fungi, the only organisms to biodegrade wood, may soon be used to biodegrade toxic chemicals as well.

into the role of lignin peroxidases in the metabolism of chemicals requiring reductive dechlorination.

Molecular oxygen can also be reduced by the oxalate radical to give superoxide, a species of oxygen that is useful for either oxidations or reductions. At low pH, superoxide is an excellent oxidant. Alternatively, superoxide is a good reductant, especially at higher pH, for reductive dechlorinations.

Superoxide is also useful to generate another powerful oxidant, the hydroxyl radical. In the presence of transition metals, such as iron, superoxide can catalyze the generation of hydroxyl radicals by a sequence of reactions called the Haber-Weiss reaction.

Because white rot fungi also produce hydrogen peroxide, it may not be necessary to dismutate superoxide to produce hydrogen peroxide. In such a case, the hydroxyl radical is simply produced by the last reaction in the sequence using Fe^{2+} and hydrogen peroxide, Fenton's reagent.

The production of oxygen radicals by white rot fungi had been suggested earlier by several investigators. However, the source of oxygen radicals remained unknown. Now the involvement in the biodegradation of environmental pollutants must be elucidated. Polyaromatic hydrocarbons present a case in point. Some of these chemicals, such as in coal tar and creosote, have oxidation potentials too high to be oxidized by the lignin peroxidases. However, polyaromatic hydrocarbons are oxidized by the fungi, and there is evidence that the lignin peroxidases

are indeed involved in their oxidation. Perhaps polyaromatic hydrocarbons are being oxidized by the hydroxyl radicals.

These mechanisms may also explain why the peroxidases catalyze the depolymerization of lignin rather than polymerization, as well as dehalogenation rather than halogenation. The presence of reductants would help form reduced, not polymerized, products of radicals.

Technology Transfer

Interest in the application of white rot fungi for the bioremediation of hazardous waste sites is growing. Researchers at Utah State University have patented the application of white rot fungi for biodegradation of many environmental pollutants, and additional patents are pending for other chemicals treatable by the fungi. Other laboratories, agencies, and environmental restoration companies are also investigating this technology and other potential applications. Field trials under the EPA SITE program are being conducted under the auspices of the Risk Reduction Engineering Laboratory in Cincinnati, Ohio.

The technology, however, must not only prove feasible in the field, but it must also be cost effective compared with traditional or alternative solutions. White rot fungi are ubiquitous and are constantly recycling carbon fixed within lignin of plants.

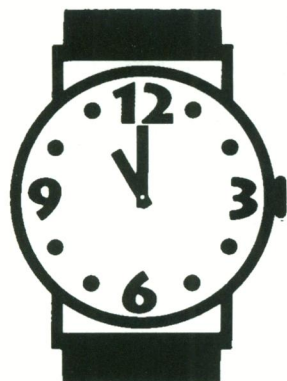
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The application of white rot fungi is expected to be relatively economical; the fungi can be grown on a number of inexpensive agricultural or forest wastes such as corn cobs and sawdust. The fungi inoculum can also be mass produced by current techniques used to produce other fungal spawn. In the quest for economical and ecologically sound methods for environmental remediation, the use of white rot fungi may not be such a rotten idea.

Steven D. Aust
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SUGGESTED READING

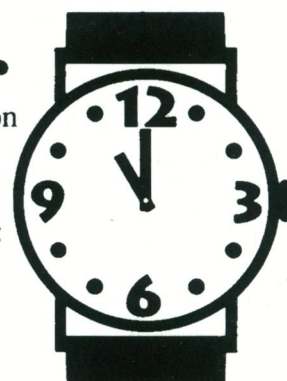
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